distribution at steady state (Vss), total plasma clearance (Cl), and plasma half-lives ($t_{1/2}$) were identical for the two enantiomers following a 10 mg/kg *iv* dose of SB-265805. The AUC and Cmax values were also equivalent for the two enantiomers. The same animals were also dosed orally at 30 mg/kg. Comparative AUC values between the oral and *iv* doses indicated that the oral bioavailability for SB-265805 was approximately 11 percent.

Plasma pharmacokinetic values were developed for beagle dogs following single 10 and 30 mg/kg *iv* doses of SB-265805. The AUC values were approximately 13 and 39 μg • hr/ml for the 10 and 30 mg/kg dose levels, respectively. The plasma half life ranged from 5 to 6 hours (compared to 2 hours for rats). The Vss and Cl values of 5 L/kg and 0.8 (L/hr)/kg were independent of dose level. Pharmacokinetics for the R- and S-enantiomers of SB-265805 were also established in dogs initially receiving a single 10 mg/kg oral dose followed by a single 10 mg/kg *iv* dose after a 4-week washout period. The AUC values for the enantiomers were similar for each dog regardless of the route of administration of SB-265805. The plasma half-life was 6 hours for each enantiomer. The AUC values following the oral dose exhibited a four-fold variation between the four animals on study, whereas the AUC values following the *iv* dose varied from animal to animal by only 28 percent. Consequently, oral bioavailibility ranged from almost 100 percent to a low of 27 percent.

Plasma protein binding for SB-265805 was 57, 59, 68, and 75 percent for mouse, dog, human, and rat plasma samples, respectively. Other *in vitro* studies with plasma indicated that interconversion of the R- and S-enantiomers did not occur during incubations under physiological conditions. *In vitro* metabolism of SB-265805 by hepatocytes from different species was evaluated after 24-hour incubations. SB-265805 was not extensively metabolized by mouse, rat, dog, and human hepatocytes. Unchanged SB-265805 plus the E-isomer of SB-265805 represented 79 to 95 percent of the original amount of SB-265805 added to each incubation. N-acetyl SB-265805 was detected in mouse, rat, and human incubations representing from 2 to 8 percent of the analyzed incubation products. N-acetyl SB-265805 was not detected in dog hepatocyte incubations. Rabbit hepatocytes metabolized SB-265805 to a greater extent with approximately 67 percent being represented by SB-265805 plus the E-isomer. Approximately 10 percent of SB-265805 in rabbit incubations was metabolized to O-desmethyl SB-265805 and 6 percent to an alcohol derivative of deaminated SB-265805 (tentative identification). N-acetyl SB-265805 was not detected in the rabbit incubation samples.

Human hepatocyte cytosolic samples were evaluated for N-acetylation activity for SB-265805 and two model substrates (p-aminobenzoic acid and sulfamethazine). A total of 46 different human hepatocyte samples were evaluated. The N-acetylation capacity for SB-265805 as substrate was limited with product formation rates ranging from 0.6 to 9 pmoles/mg protein/minute. The rate of activity for the probe substrates was approximately 3 orders of magnitude greater. Human N-acetyl transferase 2 (NAT2) activity correlated with N-acetylation of SB-265805.

The hepatocyte metabolism data indicated that SB-265805 was not readily metabolized by hepatic Cytochromes P-450. Therefore, SB-265805 should not competitively inhibit the activity of Cytochromes P-450 for different model substrates. The sponsor demonstrated that SB-265805 (as the racemate) and the purified R- and S-enantiomers did not inhibit the enzyme activity of eight subfamilies of human Cytochromes P-450 as measured by the metabolism of model substrates for each of the isozymes. In addition, the sponsor evaluated the hepatic microsomal induction capacity of SB-265805 *in vivo* with rats and dogs. Rats were dosed orally for 14 days at three different dose levels of SB-265805 (23, 67, and 159 mg/kg). SB-265805 had no effect upon hepatic microsomal protein content and overall levels of Cytochrome P-450. None of the seven evaluated Cytochrome P-450 subfamily activities were elevated (induced) as a result of SB-265805 dosing. Similar results were obtained with beagle dogs following 13 weeks of dosing at SB-265805 dose levels of 4, 24, and 96→48 mg/kg/day. Therefore, SB-265805 upon repeat dosing did not alter hepatic microsomal protein content, nmoles of Cytochrome P-450 per mg hepatic microsomal protein, and the enzymatic activities of different subfamilies of Cytochrome P-450.

The excretion and disposition of [$^{14}$C]SB-265805 were effectively evaluated in rats and dogs by the sponsor following different dosing regimens. The rates and routes of excretion were also determined in hairless mice following a single 100 mg/kg oral dose. Mice excreted 93 percent of the dosed radioactivity in feces and 3 percent in urine within 48 hours after dosing. Rats dosed orally with [$^{14}$C]SB-265805 at a dose level of 20 mg/kg excreted 73 percent of the radioactive dose in feces and 19 percent in urine. Bile-duct cannulated rats receiving the same dose eliminated 70 percent of the radioactive dose in feces, 14 percent in urine and secreted 5 percent of the radioactive dose in the bile. At a higher oral dose level of 168 mg/kg, 90 percent of the radioactive dose was eliminated in feces and 9 percent in urine. Bile-duct cannulated rats receiving the 168 mg/kg dose eliminated 86 percent of the radioactivity derived from [$^{14}$C]SB-265805 in feces, 9 percent in urine and 4 percent in bile. These data indicated that at the 20 mg/kg oral dose level to rats approximately 19 percent was systemically absorbed (urinary plus biliary percent values) compared to 13 percent absorption following the 168 mg/kg dose. However, these values may underestimate systemic absorption in rats based upon data from bile-duct cannulated rats dosed by *iv* infusion. Following a 10 mg/kg *iv* dose 33 percent of the radioactivity derived from [$^{14}$C]SB-265805 was excreted in feces, 46 percent in urine, and 12 percent was secreted in bile. The 33 percent excreted in feces was probably due to secretion of radiolabelled compounds from the intestinal epithelium in to the lumen of the G.I. tract. The data from orally dosed rats does not account for this source of fecal radioactivity which represents systemically absorbed [$^{14}$C]SB-265805.

Beagle dogs excreted 78 percent of an oral 24 mg/kg dose of [$^{14}$C]SB-265805 in feces and 14 percent in urine. Bile-duct cannulated dogs dosed at the same dose level excreted 76 percent in feces, 10 percent in urine and secreted 8 percent of the radioactive dose in bile. Bile-duct cannulated male dogs receiving a 10 mg/kg dose of [14C]SB-265805 by *iv* infusion excreted 39 percent of the radioactive dose in feces, 27 percent in urine, and secreted 30 percent in bile. Therefore, the minimum amount of systemic absorption is 18 percent of an oral dose (urine plus bile percent values). The excretion of 39 percent of an

*iv* dose in feces from bile-duct cannulated dogs, as with rats, indicated substantial potential for secretion of radioactive compounds from intestinal epithelium into the lumen of the G.I. tract (39 percent of the original *iv* dose). An accurate estimation of bioavailibility following an oral dose needs to account for secretion of SB-265805 and its metabolites from intestinal epithelium into the lumen of the G.I. tract.

Metabolite profiles were generated from plasma, bile, urine, and fecal extract samples from rats and dogs. SB-265805 was the most prominent component in fecal extracts and urine samples from all species. The relative abundance of SB-265805 plus the E-isomer of SB-265805 was typically 75 percent of the ——-analyzed radioactivity in fecal extracts and 80 percent of the urinary radioactivity from both rats and dogs. It is unclear whether formation of the E-isomer of SB-265805 is enzymatically or chemically mediated but it is present in urine, feces, bile, and plasma. The acyl glucuronide of SB-265805 was present in urine at a relative abundance of approximately 2 and 5 percent for rats and dogs, respectively. N-acetyl SB-265805 represented approximately 4 percent of the urinary radioactivity and 7 percent of the radioactivity in fecal extracts from rats. Dogs are poor acetylators consequently no N-acetyl SB-265805 was detected in urine, bile, plasma, or fecal extracts from dogs. A minor amount of O-desmethyl SB-265805 was detected in dog urine (1 to 3 percent) but not in fecal extracts. No O-desmethyl metabolite was detected in any biological sample from rats. A small amount of deaminated SB-265805 was detected in fecal extracts from both rats and dogs.

Although the acyl glucuronide metabolite of SB-265805 was not detected in fecal extracts this metabolite represented approximately 30 and 20 percent of the biliary radioactivity in rats and dogs, respectively. Because the acyl glucuronide of SB-265805 was not observed in fecal extracts from both species it is probable that glucuronidase enzymes in the gut microflora cleaved the glucuronic acid conjugate leaving SB-265805 for fecal elimination. SB-265805 and its E-isomer represented 17 and 38 percent of the biliary radioactivity in bile from rats and dogs, respectively. Deaminated SB-265805 was observed in bile from rats and dogs (approximately 3 to 4 percent relative abundance). Approximately 7 percent of the ——analyzed rat biliary radioactivity was N-acetyl SB-265805 (not seen in bile from dogs) and 8 percent of the ——analyzed dog biliary radioactivity was O-desmethyl SB-265805 (not seen in bile from rats). There were other metabolites in bile from rats and dogs but the structural identity was tentative due to the absence of authentic standards for confirmation.

The relative abundance of the E-isomer of SB-265805 in plasma from both rats and dogs ranged from 5 to 13 percent (depending upon the dose level and blood timepoint). The acyl glucuronide of SB-265805 consistently ranged from — percent of the rat or dog plasma radioactivity in the timepoints analyzed and a similar range of percent values was observed for deaminated SB-265805. N-acetyl SB-265805 represented approximately 3 percent of the rat plasma radioactivity.

The metabolism data indicated that SB-265805 was not extensively metabolized by rats and dogs. The metabolism that was confirmed by ——— was primarily direct glucuronide conjugation, deamination, or N-acetylation. None of these metabolites are

formed by Cytochrome P-450 activity and the *in vitro* metabolism data with hepatocytes also indicated that SB-265805 was not a substrate for Cytochrome P-450 activity. The only problematic *in vivo* metabolism data in the rat and dog studies concerned the extraction efficacy for the fecal extraction procedure. The data as presented by the sponsor indicated that less than 80 percent of the radioactive compounds present in fecal samples was extracted and ——-analyzed. Additionally, as much as 15 percent of the plasma radioactivity was not extracted for ——analysis. The plasma extraction procedure was essentially quantitative for SB-265805, therefore non-extracted plasma radioactivity could have been known SB-265805 metabolites or possibly macromolecularly bound metabolites.

**KEYWORDS:** Pharmacokinetics, Metabolism and Excretion, SB-265805 Metabolites

[ /S/ ] 10/16/00
Stephen G. Hundley, Ph.D.

concurrences:
HFD-590 / R. Albrecht / DDDir [ /S/ 10/26/00
HFD-590 / K. Hastings / TL 10/18/00
Disk:
HFD-590 / K. Hastings
cc:
HFD-590 / Original IND
HFD-590 / Division File
HFD-345
HFD-590 / PM /L. Kimzey
HFD-520/ Pharm/ A. Ellis
HFD-590 / MO / J. Powers
HFD-590/ MO / E. Cox
HFD-590 / Pharm / S. Hundley
HFD-590 / Micro /
HFD-590 / Biopharm /
HFD-590 / Chem /
HFD-590 / Stat /

APPEARS THIS WAY
ON ORIGINAL

DRAFT

NDA 21,158-000/Factive (gemifloxacin)

**Review and Evaluation of Pharmacology and Toxicology Data**
**Division of Anti-Infective Drug Products, HFD-520**
**CONSULTATION FOR HFD-590**

**NDA#:** 21,158-000

**Date CDER Received/Type of Submission:** 12/16/99; original NDA submission
**Reviewer:** Amy L. Ellis, Ph.D.
**Date Assigned:** 12/17/99
**Number of Volumes:** 241; 39 for pharm/tox
**Date Review Started:** 1/11/00
**Date 1ST Draft Completed:** 11/9/00
**Scientific Literature Reviewed:** No

APPEARS THIS WAY
ON ORIGINAL

**KEY WORDS:** fluoroquinolone, Factive, gemifloxacin, liver, kidney, cardiac, genotoxic, antigenic, phototoxicity, oral

**Sponsor:** SmithKline Beecham Pharmaceuticals
One Franklin Plaza
P.O. Box 7929
Philadelphia, PA 19101
(215) 751-4000; FAX (215) 751-3400

**Manufacturer:** LG Chemical Ltd.
Iksan Factory
599 Youngjei-dong, Iksan City
Chunbuk-do 570-350, Korea

APPEARS THIS WAY
ON ORIGINAL

**Review Contains Information to be Communicated to Sponsor:** Yes. The review contains labeling recommendations that have been conveyed to the sponsor already and a recommendation for a phase 4 commitment to compare gemifloxacin with other marketed fluoroquinolones in a rodent micronucleus assay with toxicokinetics.

**Submission Contains Any Integrated Tox Study Summaries in Lieu of Final Reports:** No

**Drug Information:**

**Class:** Fluoroquinolone anti-infective
**Code Names:** SB 265805-S; LB20304a
**Generic Name:** gemifloxacin
**Trade Name:** Factive
**Chemical Name:** (±)-7-(3-aminomethyl-4-(Z)-methoxyimino-1-pyrrolidinyl)-1-

NDA 21,158-000/Factive (gemifloxacin)

cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulfonate

**Structure:**

**Relevant INDs/NDAs/DMFs:** INDs —— , (oral) and —— (IV); DMF ——

**Indications:** One 320 mg tablet of Factive will be given daily for the treatment of infections caused by susceptible strains of organisms including community acquired pneumonia (7 days), acute exacerbation of chronic bronchitis (5 days), —— ——

**Clinical Formulation:**

Each tablet of Factive contains:

| | |
|---|---|
| Gemifloxacin mesylate | 426.39 mg (equivalent to 320 mg of free drug) |
| Microcrystalline Cellulose | [ |
| Povidone | |
| Crospovidone | |
| Magnesium Stearate | ] |

**Route of Administration:** Oral

**Introduction and Drug History:** Gemifloxacin is a fluoroquinolone antimicrobial and an inhibitor of bacterial DNA gyrase. Like many of the newer quinolones, it can be administered once daily and has a broader spectrum of antimicrobial activity than the older drugs in this class (active against gram positive organisms as well as gram negative). The phototoxic potential of gemifloxacin appears lower than some of the other quinolones, also a feature of several of the newer members of this class. Gemifloxacin has been shown to prolong the QTc interval of the EKG in dogs. Although the sponsor purchased the rights to gemifloxacin from LG Chemical (Korea), most of the nonclinical toxicity studies conducted with gemifloxacin have been performed in the UK by SKB or a contractor.

**Studies reviewed within this submission:**

**Safety Pharmacology Studies:**

**SB 265805: Irwin Profile in Mice** (SB Document No. SB-265805/RSD-1010VP/1)

**SB 265805: Spontaneous Locomotor Activity in Mice** (SB Document No. SB-265805/RSD-1010VR/1)

**SB 265805: Hexobarbital Sleeping Time in Mice** (SB Document No. SB-265805/RSD-1010VS/1)

**SB 265805: Anticonvulsant Activity in Mice** (SB Document No. SB-265805/RSD-1010VT/1)

**SB 265805: Single Oral Dose Cardiovascular Study in Dogs** (SB Document No. SB-265805/RSD-100Z7S/1)

**SB 265805: Single Oral Dose Cardiovascular Study in Dogs** (SB Document No. SB-265805/RSD-1010XF/1)

**SB 265805: Single Intravenous Dose Cardiovascular Study in Dogs** (Protocol G98520) (SB Document No. SB-265805/RSD-1011LN/1)

**SB 265805: Effects on Cardiac Action Potential Recorded from Dog Purkinje Fibres** (SB Document No. SB-265805/RSD-100XJS/2)

**SB 265805: Renal Function in Rats** (SB Document No. SB-265805/RSD-1010VV/1)

**SB 265805: Single Oral Dose Respiratory Study in Rats** (SB Document No. SB-265805/RSD-10110T/1)

**SB 265805: Isolated Guinea Pig Ileum** (SB Document No. SB-265805/RSD-1010VW/1)

**Acute Toxicity Studies:**

**SB 265805: Maximum Tolerated Oral Dose Study in Dogs** (SB Document No. SB-265805/RSD-100Z67/1)

**Repeat-Dose Toxicity Studies:**

**SB 265805: 4-Day Intravenous Dose Toxicity Study in Rats** (SB Document No. SB-265805/RSD-100TSJ/4)

**SB 265805: 14-Day Intravenous Dose Toxicity Study in Male Rats** (SB Document No. SB-265805/RSD-100V33/2)

**SB 265805: 1-Month Intravenous (1-Hour Infusion) Dose Toxicity Study in Rats Followed by a 28-Day Off-Dose Period** (SB Document No. SB-265805/RSD-100XTB/2)

**SB 265805: Toxicokinetics Followed by a 14-Day Fixed Dose Oral (Capsule Administration) Toxicity Study in the Dog** (SB Document No. SB-265805/RSD-1013RW/1)

**SB 265805: 13-Week Oral (Capsule Administration) Toxicity Study in the Dog** (SB Document No. SB-265805/RSD-100Z4L/1)

**SB 265805: 26-Week Oral (Capsule Administration) Toxicity Study in the Dog** (SB Document No. SB-265805/RSD-100X0R/2)

**SB 265805: 14-Day Intravenous Dose Toxicity Study in Male Dogs** (SB Document No. SB-265805/RSD-100TT5/1)

**SB 265805: 28-Day Intravenous (1-Hour Infusion) Dose Toxicity Study in Dogs** (SB Document No. SB-265805/RSD-100ZRV/2)

**Reproductive Toxicology Studies:**

**SB 265805: Oral (Gavage) Rat Developmental Toxicity Dose Ranging Study** (SB Document No. SB-265805/RSD-100NT6/1)

**SB 265805: Rat Oral (Gavage) Fertility and Embryonic Development Study** (SB Document No. SB-265805/RSD-100NT5/2)

**Quantification of LB20304 in Rat Serum Samples from a Rat Oral (Gavage) Fertility and Embryonic Development Study** (SB Document No. SB-265805/RSD-100TT7/1)

**SB 265805: Oral Dose Range Study for Effects on Embryo-Fetal Development in Mice** (SB Document No. SB-265805/RSD-100V0X/1)

**SB 265805: Oral Study for Effects on Embryo-Fetal Development in Mice** (SB Document No. SB-265805/RSD-100V0J/1)

**SB 265805: Intravenous Maximally Tolerated Dose Study in Rabbits** (SB Document No. SB-265805/RSD-100Z9X/1)

**SB 265805: An Intravenous Infusion Maximum Tolerated Dose Study in the Female Rabbit** (SB Document No. SB-265805/RSD-10110M/1)

**SB 265805: Intravenous Infusion Dose Range Study for Effects on Embryo-Fetal Development in Rabbits** (SB Document No. SB-265805/RSD-10110R/1)

**SB 265805: Intravenous Infusion Study for Effects on Embryo-Fetal Development in Rabbits** (SB Document No. SB-265805/RSD-10110S/2)

**SB 265805: Oral Study for Effects on Pre- and Postnatal Development in Rats** (SB Document No. SB-265805/RSD-10110P/2)

**Genetic Toxicology Studies:**

**Induction of** [redacted] **in the Presence of** [redacted] (SB Document No. SB-265805/RSD-100X0S/3)

**SB-265805-S: A Study to Investigate Effects on Bone Marrow Following Oral Administration to Rats** (SB Document No. SB-265805/RSD-1014PP/1)
**Intravenous Micronucleus Assay in Rats** (SB Document No. SB-265805/RSD-10110J/1)

**Special Toxicity Studies:**

**Generation of Anti SB-265805-S Antisera in the Guinea Pig** (SB Document No. SB-265805/RSD-10110F/2)

**SB 265805: 13-Week Oral (Gavage) Subchronic Study in Hairless Mice, With or Without Added Simulated Sunlight** (SB Document No. SB-265805/RSD-10110V/1)

**SB 265805: Oral (Gavage) Toxicokinetic Study in Hairless Mice** (SB Document No. SB-265805/RSD-100ZTZ/2)

**SB 265805: Four-Week Oral (Gavage) Toxicokinetics Study in Hairless Mice** (SB Document No. SB-265805/RSD-10110N/1)

**SB 265805: 12-Month Oral (Gavage) Study to Determine the Influence on Photocarcinogenesis in Hairless Mice** (SB Document No. SB-265805/RSD-1014WP/1)

**Microscopic Examination of Liver From DMPK Study No. 6146-205: "Biliary and Plasma Concentrations of Drug-Related Material Following Single and 5-Day Repeat Administration of [14C]SB-265805-S to Male Bile Duct-Cannulated Beagle Dogs at a Target Dose of 30 mg Free Base/kg/day** (SB Document No. SB-265805/RSD-1014X3/1)

**X-Ray Energy Spectroscopy of Bile Duct Inclusions from a Dog Dosed with SB-265805-S (I99544)** (SB Document No. SB-265805/RSD-1014XF/1)

**Solubility of SB-265805 (free base) in Gallbladder and Hepatic Dog Bile** (SB-265805/RSD-1014S7/2)

**Studies not reviewed in this document (and location of review):**

The following Pharmacokinetic Studies will be reviewed by Dr. Stephen Hundley and filed under the current NDA:

**An Investigatory Study to Examine Extraction Methodology for SB-265805 and Drug-Related Material From Biological Matrices** (SB Document No. SB-265805/RSD-100ZBR/1)

**Summary Report, Preparation of Isotopically Labeled SB 265805-S** (SB Document No. SB 265805/RSD-101446/1)

**Determination of SB-265805 in Mouse Plasma by** ——— (SB Document No. SB-265805/RSD-100RVF/1)

**Validation of an ——Method with ——— Detection for the Quantification of LB20304 in Rat Serum** (SB Document No. SB-265805/RSD-100WFV/1)

**Determination of SB-265805 in Rat Plasma by** ——— (SB Document No. SB-265805/RSD-100MPZ/1)

**Determination of SB-265805 (R,S) Enantiomers in Rat Plasma by** ——— (SB Document No. SB-265805/RSD-100RK5/2)

**Determination of SB-414000 (N-acetyl SB-265805) in Rat Plasma by** ——— (SB Document No. SB-265805/RSD-1010XC/1)

**Determination of SB-265805 in Rat Serum by** ——— (SB Document No. SB-265805/RSD-100ZPX/1)

**Determination of SB-265805 in Rabbit Plasma by** ——— (SB Document No. SB-265805/RSD-100MPX/2)

**Determination of SB-265805 in Dog Plasma by** ——— (SB Document No. SB-265805/RSD-100MZG/1)

APPEARS THIS WAY
ON ORIGINAL

NDA 21,158-000/Factive (gemifloxacin)

**Determination of SB-265805 (R,S) Enantiomers in Dog Plasma by** [redacted] (SB Document No. SB-265805/RSD-100RK6/1)

**Plasma Concentrations and Excretion of Drug-Related Material Following a Single Oral Administration of [14C]SB-265805-S to Male and Female Hairless Mice at a Target Dose of 100 mg Free Base/kg** (SB Document No. SB-265805/RSD-1011R3/1)

**A Study to Determine the Pharmacokinetics of SB-265805 Following Intraperitoneal Administration of SB-265805-S at a Nominal Dose Level of 50 mg/kg (as the Mesylate Salt) in the Mouse** (SB Document No. SB-265805/RSD-100XLK/1)

**A Preliminary Study to Characterize Drug-Related Material in Urine, Bile, Faeces, and Plasma Following a Single Oral Administration of [14C]SB-265805-S to the Male Rat at a Target Dose Level of 20 mg Free Base/kg, and to Assess Excretion of Compound Related Material Following a Single Intravenous Administration at a Target of 10 mg Free Base/kg** (SB Document No. SB-265805/RSD-100TVN/2)

**Elimination of Drug-Related Material Following Single Doses of [14C]SB-265805-S to Male and Female Rats** (SB Document No. SB-265805/RSD-100XCS/1)

**An Investigative Study to Determine the Pharmacokinetics of the R-and S-Enantiomers of SB-265805 in the Rat Following Both Intravenous and Oral Administration of Racemic RS-SB-265805-S at Nominal Dose Levels of 10 and 30 mg Free Base/kg, Respectively** (SB Document No. SB-265805/RSD-100TS0/1)
**A 14-Day Oral Study in Rats to Investigate the Pharmacokinetics of SB-265805 and the Effect on Hepatic Levels of Cytochrome P450 and Related Parameters** (SB Document No. SB-265805/RSD-100V86/3)

***In Vitro* Stability and *In Vivo* Interconversion Study** (SB Document No. SB-265805/RSD-10108L/1)

**SB-265805 (Gemifloxacin): A Study to Determine the Pharmacokinetics of Gemifloxacin and SB-414000 (N-acetyl Gemifloxacin) Following Oral Administration of Gemifloxacin Mesylate at Nominal Doses of 210 and 750 mg/kg (as the Mesylate Salt) in the Rat** (SB Document No. SB-265805/RSD-1013DJ/1)

**Biliary and Plasma Concentrations of Drug-Related Material Following Single and 5-Day Repeat Intravenous Administration of [14C]SB-265805-S to Male Bile Duct-Cannulated Beagle Dogs at a Target Dose of 30 mg Free Base/kg/Day** (SB Document No. SB-265805/RSD-1011R4/1)

**An Investigation of the Plasma Concentrations of SB-265805 and the Biliary Metabolite Profiles Following Single and 5-Day Repeat Intravenous Administration**

**of [14C]SB-265805-S to Male Bile Duct-Cannulated Beagle Dogs at a Target Dose of 30 mg Free Base/kg/Day** (SB Document No. SB-265805/RSD-1011XJ/1)

**A Preliminary Study to Investigate the Absorption, Excretion, and Biliary Secretion of Drug-Related Material in the Dog Following a Single Oral Administration of [14C]SB-265805-S at a Target Dose of 24 mg Free Base/kg** (SB Document No. SB-265805/RSD-100SKR/2)

**An Investigative Study to Determine the Pharmacokinetics of the R-and S-Enantiomers of SB-265805 Following Both Intravenous and Oral Administration of SB-265805-S at a Nominal Dose Level of 10 mg/kg (as the Free Base) to the Male Beagle Dog** (SB Document No. SB-265805/RSD-100T6Z/1)

**A Study in the Beagle Dog to Determine the Intravenous Pharmacokinetics of SB-265805 Following a 30 Minute Intravenous Infusion of SB-265805-S at Nominal Dose Levels of 10 and 30 mg/kg (as the Free Base)** (SB Document No. SB-265805/RSD-100WK7/1)

**A Study in the Beagle Dog to Determine the Concentrations in Cardiac Tissue Following a 30 Minute Intravenous Infusion of SB-265805-S at a Nominal Dose Level of 30 mg/kg (as the Free Base)** (SB Document No. SB-265805/RSD-1013MS/1)

**The *In Vitro* Blood/Plasma Partitioning of [14C]SB-265805 in Rat, Dog, and Man** (SB Document No. SB-265805/RSD-100XCN/2)

**The *In Vitro* Protein Binding of the R and S Enantiomers of SB-265805-S in Rat, Mouse, Dog, and Human Plasma** (SB Document No. SB-265805/RSD-100T54/1)
**[14C]SB-265805-S: Quantitative Whole-Body Autoradiography Following Single Oral Administration (210 mg/kg) to the Male Pigmented Rat** (SB Document No. SB-265805/RSD-100XCM/1)

**Placental Transfer of Drug-Related Material Following a Single Oral Administration of [14C]SB-265805-S to the Pregnant Rat at a Nominal Dose Level of 270 mg/kg (217 mg Free Base/kg)** (SB Document No. SB-265805/RSD 100XCP/2)

**Milk Secretion of Drug-Related Material Following a Single Oral Administration of [14C]SB-265805-S to the Lactating Rat at a Nominal Dose Level of 30 mg/kg (24 mg Free Base/kg)** (SB Document No. SB-265805/RSD 100XCR/1)

**The Effect of SB-265805-S on Hepatic Levels of Cytochrome P450 and Related Parameters in Beagle Dogs After Oral Administration at 0, 5, 30 and 120 mg/kg/day for 13 Weeks** (SB Document No. SB-265805/RSD-100XG0/2)

**A Preliminary Investigation Into the Cytochrome P450 Inhibitory Potential of SB-265805** (SB Document No. SB-265805/RSD-10158N/1)

**Evaluation of Racemic SB-265805 and Its Individual Enantiomers as Inhibitors of Human P450 Enzymes *in Vitro*** (SB Document No. SB-265805/RSD-100ZHK/2)

**The Metabolism of [14C]SB-265805 in the Hairless Mouse** (SB Document No. SB-265805/RSD-1011XK/1)

**Biotransformation of [14C]SB-265805 in Rat and Dog** (SB Document No. SB-265805/RSD-100XFZ/1)

**Characterisation of the Metabolism of [14C]SB-265805 in Whole Cell Liver Tissue Models** (SB Document No. SB-265805/RSD-100W5S/1)

**A Preliminary *in Vitro* Investigation into the Biotransformation of SB-265805** (SB Document No. SB-265805/RSD-100XFX/1)

**An *in Vitro* Investigation into the N-acetylation of SB-265805** (SB Document No. SB-265805/RSD-1013M3/1)

**The Metabolism of [14C]SB-265805 in Whole Liver Cell Systems from Rabbit and Mouse** (SB Document No. SB-265805/RSD-100XCV/1)

**Elimination of Drug-Related Material Following Single Doses of [14C]SB-265805-S to Male and Female Dogs** (SB Document No. SB-265805/RSD-100XCK/1)

**Investigation of SB-265805 (Fluoroquinolone) Permeability *in Vitro* Across Various Intestinal Models** (SB Document No. SB-265805/RSD-1010V9/1)
The remaining studies were reviewed previously by Dr. Amy Ellis and filed as noted:

**Safety Pharmacology Studies:**

**Safety Pharmacology of LB20304a** (Study No. B-97-0038-P); IND [redacted]

**The Effects of LB20304a on the Hemodynamics and Cardiac Function of Anesthetized Beagle Dogs Following a Single Intravenous Dose** (Study No. 652-95); IND [redacted]

**SB 265805: Single Intravenous Dose Cardiovascular Study in Dogs** (SB Document No. SB-265805/RSD-100THH/1); IND [redacted]

NDA 21,158-000/Factive (gemifloxacin)

**Pharmacokinetic Studies:**

**Validation of the Bioanalytical Method for the Measurement of LB20304a in Rat Plasma** (Study No. VPKA-001); IND

**Validation of the Analytical Procedure for the Determination of LB20304a in Rat Serum Using with Detection** (Study No. 1405/14-1010); IND

**Validation of the Bioanalytical Method for the Measurement of LB20304a in Dog Plasma** (Study No. VPKA-001; SB Document No. SB-265805/RSD-100MB0/3); IND

**Validation of the Bioanalytical Method for the Measurement of LB20304a in Dog Plasma** (Study No. VPKA-002); IND

**Validation of the Analytical Procedure for the Determination of LB20304a in Dog Serum Using with Detection** (Study No. 1405/13-1010); IND

**$^{14}$C-LB20304a- Pharmacokinetics, Tissue Distribution, Metabolism, and Excretion Studies in the Rat** (Study No. LKY 50/962743); IND

**LB20304: Pharmacokinetics in Rats and Dogs** (Study No. PKL B20304-3); IND

**LB20304: Metabolism** (Study No. PKL B20304-4); IND

**LB20304: Solubility and Stability** (Study No. PKL B20304-5); IND

**Acute Toxicity Studies:**

**Single Dose Oral (Gavage) Toxicity Study in the Rat** (Study No. 1405/1-1032); IND

**Single Dose (Intravenous) Toxicity Study in the Rat** (Study No. 1405/2-1032); IND

**Single Dose Oral (Gavage) Toxicity Study in the Mouse** (Study No. 1405/3-1032); IND

**Single Dose (Intravenous) Toxicity Study in the Mouse** (Study No. 1405/4-1032); IND

**SB 265805: Maximum Tolerated Intravenous Dose Study in Dogs** (SB Document No. SB-265805/RSD-100RCW/1; IND [redacted]

**SB 265805: Maximum Tolerated Intravenous Infusion Study in Male Dogs** (SB Document No. SB-265805/RSD-100T9S/1; IND [redacted]

**SB 265805: Maximum Tolerated Intravenous Dose Toxicity Study in Dogs (Batch No. [redacted]-A-03C)** (SB Document No. SB-265805/RSD-100TFC/1; IND [redacted]

**Repeat-Dose Toxicity Studies:**

**LB-20304a: 7 Day Oral (Gavage Administration) Toxicity Study in the Rat** (Study No. 1405/5-1050); IND [redacted]

**LB-20304a: 28 Day Oral (Gavage Administration) Toxicity Study in the Rat With a 14 Day Treatment-Free Period** (Study No. 1405/7-1050); IND [redacted]

**LB-20304a: 13-Week Oral (Gavage Administration) Toxicity Study in the Rat** (SB Document No. SB-265805/RSD-100LW4/1); IND [redacted]

**LB-20304a: Maximum Tolerated Dose (MTD) Followed by a 14 Day Fixed Dose Oral (Capsule Administration) Toxicity Study in the Dog** (Study No. 1405/6-1050); IND [redacted]

**LB-20304a: 28 Day Oral (Capsule Administration) Toxicity Study in the Dog** (Study No. 1405/8-1050); IND [redacted]

**SB 265805: 13-Week Oral (Capsule Administration) Toxicity Study in the Dog With a 4-Week Treatment-Free Period** (SB Document No. SB-265805/RSD-100LW5/2); IND [redacted]

**SB 265805: 4-Day Intravenous Dose Range Study in Dogs** (SB Document No. SB-265805/RSD-100SJF/2); IND [redacted]

**SB 265805: 1-Month Intravenous Dose Toxicity Study in Dogs** (SB Document No. SB-265805/RSD-100TG4/1); IND [redacted]

**Reproductive Toxicology Studies:**

**SB 265805: Oral Maximally Tolerated Dose Study in Rabbits** (SB Document No. SB-265805/RSD-100RB5/2); IND [redacted]

**Genetic Toxicology Studies:**

APPEARS THIS WAY
ON ORIGINAL

**LB20304a: Reverse Mutation in Four Histidine-Requiring Strains of *Salmonella Typhimurium*** (Study No. S017); IND [redacted]

**LB 20304a: Induction of Micronuclei in the Bone Marrow of Treated Mice** (Study No. 1405/11-1152); IND [redacted]

**LB 20304a: Mutation at the Thymidine Kinase (tk) Locus of Mouse Lymphoma L5178Y Cells Using the [redacted] Technique** (Study No. 1405/9-1052); IND [redacted]

**LB 20304a: Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes** (Study No. 1405/10-1052); IND [redacted]

**LB 20304a: Measurement of Unscheduled DNA Synthesis in Rat Liver Using an *In Vivo/In Vitro* Procedure** (Study No. 1405/12-1052); IND [redacted]

**Special Toxicity Studies:**

**LB 20304a: Assessment of Antigenicity in the Guinea Pig (Active Anaphylaxis Test)** (Study No. LKY 39/961625); IND [redacted]

**LB 20304a: Assessment of Antigenicity in the Mouse (Passive Cutaneous Anaphylaxis Test)** (Study No. LKY 40/961987); IND [redacted]

**LB 20304a: Assessment of Antigenicity in the Mouse (Passive Hemaglutination Test)** (Study No. LKY 41/961988); IND [redacted]

**Phototoxicity Studies of LB20304a** (Study No. P019); IND [redacted]

APPEARS THIS WAY
ON ORIGINAL

**REVIEWS:**

Unless otherwise specified, the doses of gemifloxacin used in most of the nonclinical studies have been expressed in terms of the mesylate salt.

NDA 21,158-000/Factive (gemifloxacin)

## SAFETY PHARMACOLOGY

**SB 265805: Irwin Profile in Mice** (Protocol No. G99545; SB Document No. SB-265805/RSD-1010VP/1)

Report dated 8/24/99, —and —— GLP

Vol. 1.5.004

**Summary:** Male CD-1 mice (6/group) were fasted for 2 hours then given a single 25, 100, 250, or 450 mg/kg oral doses of gemifloxacin mesylate (Batch No. EF03-14R1P5) at a dose volume of 10 ml/kg. Vehicle control animals received 10 ml/kg of 0.9% saline and comparator animals received 1 mg/kg of clonidine. The mice were evaluated according to Irwin profile parameters for behavior and appearance 30, 90, 150, and 300 minutes after dosing.

No drug-related changes in the Irwin profile were observed in mice given up to 250 mg/kg of gemifloxacin. Changes in the 450 mg/kg group that appeared related to gemifloxacin were slight, transient reductions in respiratory rate and muscle tone. Clonidine administration produced the expected effects (decreased alertness and activity, abnormal gait, decreased muscle tone, slow respiration, reduced startle response, etc.).

**SB 265805: Spontaneous Locomotor Activity in Mice** (Protocol No. G99546; SB Document No. SB-265805/RSD-1010VR/1)

Report dated 8/24/99, — and ——GLP

Vol. 1.5.004

**Summary:** Male CD-1 mice (8/group) were fasted for 2 hours then given a single 25, 100, 250, or 450 mg/kg oral doses of gemifloxacin mesylate (Batch No. EF03-14R1P5) at a dose volume of 10 ml/kg. Vehicle control animals received 10 ml/kg of 0.9% saline and comparator animals received 20 mg/kg of chlorpromazine or 10 mg/kg amphetamine. The mice were placed in individual observation cages with infrared beam activity meters 30 and 90 minutes after dosing and monitored for 9 minutes.

No changes in total locomotor or ambulatory activity were observed after the 25 or 100 mg/kg doses of gemifloxacin. Statistically significant reductions in both locomotor and ambulatory activity were observed after either the 250 or 450 mg/kg dose of gemifloxacin at both the 30 and 90 minute observation time points. As expected, chlorpromazine was associated with reduced activity and amphetamine with increased

activity.

**SB 265805: Hexobarbital Sleeping Time in Mice** (Protocol No. G99547; SB Document No. SB-265805/RSD-1010VS/1)

[redacted]

Report dated 10/5/99, [redacted] and [redacted] GLP

Vol. 1.5.004

**Summary:** Male CD-1 mice (8/group) were fasted for 2 hours then given a single 25, 100, 250, or 450 mg/kg oral doses of gemifloxacin (Batch No. EF03-14R1P5) at a dose volume of 10 ml/kg. Vehicle control animals received 10 ml/kg of 0.9% saline and comparator animals received 20 mg/kg of chlorpromazine. One hour after oral dosing, each mouse received 80 mg/kg of hexobarbital via the intraperitoneal route. The time from the loss to the regaining of the righting reflex was noted and sleeping time was determined.

Pretreatment of the mice with gemifloxacin at doses up to 450 mg/kg did not either time to loss of the righting reflex or sleeping time following hexobarbital injection. As expected, chlorpromazine potentiated the hexobarbital-induced sleeping time in the mice.

**SB 265805: Anticonvulsant Activity in Mice** (Protocol No. G99548; SB Document No. SB-265805/RSD-1010VT/1)

[redacted]

Report dated 10/5/99, [redacted] and [redacted] GLP

Vol. 1.5.004

**Summary:** Male CD-1 mice (8/group) were fasted for 2 hours then given a single 25, 100, 250, or 450 mg/kg oral doses of gemifloxacin (Batch No. EF03-14R1P5) at a dose volume of 10 ml/kg. Vehicle control animals received 10 ml/kg of 0.9% saline and comparator animals received 20 mg/kg of [redacted] One half hour after oral dosing, each mouse received 85 mg/kg of [redacted] subcutaneously and was observed for 45 minutes for convulsions.

Gemifloxacin had no anticonvulsant activity. The incidence of tonic convulsions was greater in all of the gemifloxacin groups than vehicle control mice (4-5/8 instead of 1/8), though the difference was not statistically significant. The incidence of clonic convulsions (7-8/8) and the time to first convulsion (about 5-12 minutes) were similar in gemifloxacin groups and vehicle control. The positive control article [redacted]

had significant anticonvulsant activity (no pentylenetetrazole-induced convulsions were observed in these mice) as would be expected.

**SB 265805: Single Oral Dose Cardiovascular Study in Dogs** (SB Document No. SB-265805/RSD-100Z7S/1; Protocol No. G98615)

B. Gascoyne, A. Pritchard, A. Porter (SmithKline Beecham, The Frythe, Welwyn, Herts, UK)

Report dated 6/24/99, UK, US, and OECD GLP

Vol. 1.5.004

**Animals:** Beagle dogs, 2/sex, 4.3-7.5 years old, 14.2-17.5 kg

**Diet:** ——— Dog Maintenance Diet 9682 (E) SQC Lab diet A was provided daily. Dogs were fasted for about 18 hours prior to drug treatment. Filtered tap water was provided *ad libitum*.

**Drug Dose and Route of Administration:** The doses of gemifloxacin used in this study were 50, 100, and 200 mg/kg. Gemifloxacin mesylate powder (Batch # ——A-07C) was put into gelatin capsules and administered orally to the dogs. Empty gelatin capsules were used as a placebo control.

**Length and Conduct of Study:** On separate testing days (at least one week apart), placebo or gelatin capsules containing gemifloxacin were administered to the dogs. ECG, blood pressure, and heart rate were monitored prior to dosing and for 6 hours after administration of gemifloxacin. The dogs were conscious, but restrained in Pavlov slings during the dosing and measurement of cardiac parameters. Blood samples for toxicokinetic analysis were collected before drug was given and 0.5, 1, 2, 4, 6, and 24 hours after dosing. Plasma levels of gemifloxacin were measured using — with ——— and the lower limit of detection was —— g/ml.

**Results:** Vomiting was observed in one male after 50 mg/kg gemifloxacin, in both males after 100 mg/kg, and in all four dogs following the 200 mg/kg dose. Salivation was noted in one dog after the administration of 200 mg/kg. Lip licking, restlessness, and panting were observed in some animals after all gemifloxacin doses.

Changes in heart rate and blood pressure (systolic, diastolic, mean) were not observed following gemifloxacin dosing. The QTc interval did not change following any of the gemifloxacin doses, but the QRS complex duration increased following all doses of the drug. The increases were not dose-dependant (6, 15, and 10 msec at 50, 100, and 200 mg/kg, respectively) and were observed beginning at about 1.3 hours and lasting

until 4.7 hours after administration of the low gemifloxacin dose and starting 2-3 hours after the higher doses were given and lasting throughout the monitoring period. No other changes in the ECG (intervals or amplitudes) were observed.

Gemifloxacin plasma levels were above the level of detection throughout the 24 hour sampling period. Cmax and AUC increased with dose, but the increases were not dose proportional.

**Mean (± SD) Pharmacokinetic Parameters Following Oral Administration of Gemifloxacin to Male and Female Dogs**

| Dose (mg/kg) | Cmax (g/ml) | $AUC_{0\text{-}24\,h}$ (gh/ml) | Median Tmax (hr) [Range] |
|---|---|---|---|
| 50 | 5.04 ± 0.43 | 41.5 ± 11.8 | 3.03 [1.08-4.07] |
| 100 | 5.12 ± 1.49 | 48.3 ± 18.5 | 5.05 [1.28-6.10] |
| 200 | 6.76 ± 2.07 | 69.7 ± 35.4 | 3.09 [2.07-6.03] |

**SB 265805: Single Oral Dose Cardiovascular Study in Dogs** (SB Document No. SB-265805/RSD-1010XF/1; Protocol No. G99537)

B. Gascoyne, A. Pritchard, J. Bullman (SmithKline Beecham, The Frythe, Welwyn, Herts, UK)

Report dated 8/5/99, UK, US, and OECD GLP

APPEARS THIS WAY ON ORIGINAL

Vol. 1.5.004

**Animals:** Beagle dogs, 2/sex, 5.1-8.3 years old, 14.1-17.9 kg (these were the same dogs that were used in the study above)

**Diet:** ——————Dog Maintenance Diet (E) SQC Lab diet A was provided daily. Dogs were fasted for about 18 hours prior to drug treatment. Filtered tap water was provided *ad libitum*.

**Drug Dose and Route of Administration:** The doses of gemifloxacin used in this study were 4, 20, and 40 mg/kg. Gemifloxacin mesylate powder (Batch # ——A-07C) was put into gelatin capsules and administered orally to the dogs. Empty gelatin capsules were used as a placebo control. The investigators had intended to use gemifloxacin doses of 5, 25, and 50 mg/kg, but the amounts of drug put into the capsules did not account for the weight of the mesylate salt.

**Length and Conduct of Study:** On separate testing days (at least one week apart), placebo or gelatin capsules containing gemifloxacin were administered to the dogs.

APPEARS THIS WAY ON ORIGINAL

ECG, blood pressure, and heart rate were monitored prior to dosing and for 6 hours after administration of gemifloxacin. The dogs were conscious, but restrained in Pavlov slings during the dosing and measurement of cardiac parameters. Blood samples for toxicokinetic analysis were collected before drug was given and 0.5, 1, 2, 3, 4, 6, and 24 hours after dosing. Plasma levels of gemifloxacin were measured using [redacted] with [redacted] and the lower limit of detection was [redacted] g/ml.

**Results:** Vomiting was observed in both males after the 40 mg/kg gemifloxacin dose. Lip licking and salivation were also noted in one of these dogs, with the former clinical sign also observed in this dog after it received 20 mg/kg. Lip licking and panting were observed in one female after 40 mg/kg gemifloxacin. Panting was seen in both females after administration of 4 mg/kg.

Changes in heart rate and blood pressure (systolic, diastolic, mean) were not observed following gemifloxacin dosing. There were no drug-related changes in ECG (including the QTc interval or the QRS complex duration) after any of the gemifloxacin doses.

Gemifloxacin plasma levels were above the level of detection throughout the 24 hour sampling period. Cmax and AUC increased in an approximately dose proportional manner between 20 and 40 mg/kg, but the increase was about twice what would have been expected based upon dose proportionality between 4 and 20 mg/kg.

**Mean (± SD) Pharmacokinetic Parameters Following Oral Administration of Gemifloxacin to Male and Female Dogs**

| Dose (mg/kg) | Cmax (g/ml) | $AUC_{0\text{-}24\,h}$ (gh/ml) | Median Tmax (hr) [Range] |
|---|---|---|---|
| **4** | 0.205 ± 0.083 | 2.03 ± 1.04 | 2.04 [0.53-4.03] |
| **20** | 2.31 ± 0.927 | 26.2 ± 12.8 | 1.08 [1.05-6.02] |
| **40** | 3.78 ± 1.07 | 44.8 ± 8.04 | 2.62 [1.07-4.03] |

**SB 265805: Single Intravenous Dose Cardiovascular Study in Dogs** (SB Document No. SB-265805/RSD-1011LN/1; Protocol No. G98520)

B. Gascoyne, A. Pritchard (SmithKline Beecham, The Frythe, Welwyn, Herts, UK)

Report dated 6/22/99, UK, US, and OECD GLP

Vol. 1.5.004

**Summary:** This study was terminated after one male beagle dog received 3 mg/kg of gemifloxacin and a second received 10 mg/kg via IV infusion over 10 minutes. No clinical signs were observed following the 3 mg/kg dose. However, clinical signs (red

ears, head shaking, facial swelling, restlessness) were much more severe than expected after the 10 mg/kg dose based upon the results of previous studies. The investigators were concerned that the physical characteristics of the dosing solution had changed. Brown deposits were observed upon filtration of the solution and they had not been seen previously. Additionally, on the first day of a one month repeat dose IV study that was being run concurrently with this experiment, clinical signs following a 10 mg/kg dose of gemifloxacin were similar to those reported here. Thus, the investigators decided to terminate the current study.

**SB 265805: Effects on Cardiac Action Potential Recorded from Dog Purkinje Fibres** (SB Document No. SB-265805/RSD-100XJS/2)

J.F. Faivre, A. Bril (SmithKline Beecham, Dept. of Cardiovascular Biology, Cedex, France)

Report issued 9/99 and amended 10/99, no GLP statement

Vol. 1.5.005

**Results:** Gemifloxacin caused a slight but statistically significant ($p \leq 0.01$) reduction in action potential amplitude only at 100 M (122.25 $\pm$ 1.93 mV for gemifloxacin vs. 123.50 $\pm$ 1.85 mV for control). The APD50 (257.75 $\pm$ 26.35 vs. 235.25 $\pm$ 32.45) and the APD90

(349.50 ± 30.66 vs. 320.75 ± 34.63) were also significantly prolonged in the presence of 100 M gemifloxacin. The mean plasma Cmax in humans after a 320 mg dose of gemifloxacin is approximately 1.5 g/ml (about 3.2 M).

[redacted] did not appear to have any effect on the [redacted] (all at 100 M) caused small, but statistically significant reductions in action potential amplitude. [redacted] caused larger reductions in maximum upstroke velocity at 100 M. There was a modest increase in APD90, but not APD50 in the presence of 100 M [redacted] Both 10 and 100 M [redacted] were associated with significant prolongations of the APD50 and APD90. The average prolongations of the APD50 and APD90 caused by [redacted] were greater than those caused by [redacted] increased the action potential duration of canine [redacted] by about 10% at 100 M, but no change occurred at 10 M. [redacted] were associated with greater increases in APD (approximately 30% and 60%, respectively, at APD90). Unlike [redacted] was not associated with a decrease in maximum upstroke velocity.

**SB 265805: Renal Function in Rats** (SB Document No. SB-265805/RSD-1010VV/1)

[redacted]

Report dated 10/19/99, [redacted] and [redacted] GLP
Vol. 1.5.004

**Summary:** Male Harlan Sprague-Dawley rats (8/group) received 20 ml/kg of sterile 0/9% saline via oral gavage. Thirty minutes later a single oral dose of gemifloxacin (Batch [redacted]) was given (10, 25, 50, or 170 mg/kg, dose volume 10 ml/kg). A vehicle control group received saline and a positive control group received 20 mg/kg of [redacted] Rats were put into individual metabolism cages and urine was collected 0-2, 2-4, 4-8, and 8-24 hours after dosing.

Compared to vehicle treated rats, the 10 mg/kg dose of gemifloxacin did not have an effect on any of the urinary parameters measured. During the 0-2 hour collection period, the rats treated with ≥25 mg/kg of gemifloxacin excreted smaller volumes of urine and less total sodium, potassium, and chloride than vehicle controls; thus, osmolality did not differ from control. The pH of the urine of these gemifloxacin groups was also slightly greater than controls (8.29 for controls vs. up to 8.99 for gemifloxacin)- the investigators thought this might have been due to the excretion of mesylate. During the 2-4 and 4-8 hour collection periods, the osmolality of the urine of rats treated with ≥25 mg/kg of gemifloxacin was higher than vehicle control; the excretion rate of potassium increased concomitantly. No change in creatinine excretion was observed in the gemifloxacin-treated rats during any of the collection periods. Over the entire 24 hour period, the average urine volume for each animal and its excretion of electrolytes did not differ between the vehicle control and gemifloxacin-treated rats. [redacted] had its characteristic diuretic effect on the rats.

**SB 265805: Single Oral Dose Respiratory Study in Rats** (SB Document No. SB-265805/RSD-10110T/1)

J.P. Renninger, D.J. Murphy (SmithKline Beecham, King of Prussia, PA)

Report dated 9/24/99, U.S. and U.K. GLP

Vol. 1.5.005

**Summary:** Male Sprague-Dawley rats (4/group) were fasted for 2 hours then given a single 50, 170, or 600 mg/kg oral doses of gemifloxacin (Batch No. EF03-14R1P5) at a dose volume of 10 ml/kg. Vehicle control animals received 10 ml/kg of 0.9% saline. The same animals were used for different doses with one week in between each dose. The rats had surgically implanted telemetry devices implanted for measuring pleural pressure and a volume displacement plethysmograph chamber was used for measuring lung volumes and airflow. Respiratory parameters (tidal volume, respiratory rate, minute volume, peak inspiratory flow, peak expiratory flow, fractional inspiratory time, and total pulmonary resistance) were measured in conscious animals prior to dosing, then 1, 2, and 4 hours after administration of drug (3-7 minutes of monitoring at each time point).

The 50 mg/kg dose of gemifloxacin had no effect on the rats' respiration. The 170 and 300 mg/kg doses were associated with a dose-dependant respiratory depression that was observed beginning 1 hour after dosing and persisted 24 hours after dosing. The 600 mg/kg dose caused decreases (about 60-90% of predose values) in minute volume tidal volume, respiratory rate, and peak inspiratory flow. The 170 mg/kg dose decreased minute volume, tidal volume and peak inspiratory flow, but not respiratory rate. None of the gemifloxacin doses was associated with changes in peak expiratory flow, fractional inspiratory time, or total pulmonary resistance.

**SB 265805: Isolated Guinea Pig Ileum** (Protocol No. G99550; SB Document No. SB-265805/RSD-1010VW/1)

Report dated 10/28/99, UK and OECD GLP

Vol. 1.5.005

**Summary:** Ileum segments were taken from male Dunkin-Hartley guinea pigs (2 segments could be obtained from each animal) and incubated in organ baths filled with [redacted] solution at 37C, 95%$O_2$, 5%$CO_2$. The segments were connected to an isometric force transducer under 1 g tension and allowed to equilibrate for 30 minutes before testing began. The contraction response of an ileum segment to acetylcholine, histamine,

5-hydroxytryptamine, or barium chloride was characterized (a dose-response curve was generated for each), then exposure to gemifloxacin (Batch No. EF03-14R1P5) concentrations of 0, 3, 10, or 100 M occurred for 30 minutes. Three or 4 segments were tested for each contracting substance with one concentration of gemifloxacin. Changes in the tone of the segments upon exposure to gemifloxacin were recorded. After gemifloxacin exposure was finished, the drug was washed out of the system and the original contracting substance was retested.

None of the gemifloxacin concentrations changed the basal tone of the ileum segments. The contractions induced by ——— and ——— (maximum force of the contractions as well as the effective doses of each compound) were not altered in a biologically significant way by any concentration of gemifloxacin.

## TOXICOLOGY

APPEARS THIS WAY
ON ORIGINAL

**Acute Toxicity Studies:**

**SB 265805: Maximum Tolerated Oral Dose Study in Dogs** (SB Document No. SB-265805/RSD-100Z67/1; Protocol No. D98614)

J.M. Birmingham, A. Pritchard, A. Porter (SmithKline Beecham, The Frythe, Welwyn, Herts, UK)

Report dated 9/3/99, not GLP

APPEARS THIS WAY
ON ORIGINAL

Vol. 1.5.006

**Animals:** One male (10.0 kg, 14 months old) and one female (9.2 kg, 22 months old) beagle dog ( ——— )

**Diet:** ——— Dog Maintenance Diet (400 g/dog) was offered daily approximately one hour after dosing and filtered tap water was available *ad libitum*.

**Drug Dose and Route of Administration/Length and Conduct of Study:**
Gemifloxacin mesylate (Batch No. ——A-07C) powder was put into gelatin capsules and administered orally. The starting dose for both dogs on day 1 of the study was 100 mg/kg. The following doses were given to each dog based on the results of the day before:

| Day | Male Dose (mg/kg) | Female Dose (mg/kg) |
|---|---|---|
| 1 | 100 | 100 |
| 2 | 50 | 50 |
| 3 | 200 | 0 (capsule containing glucose) |
| 4 | 300 | 25 |

APPEARS THIS WAY
ON ORIGINAL

| | | |
|---|---|---|
| **5** | 250 | 10 |
| **6-7** | No dose given | No dose given |
| **8 (TK profile)** | 200 | 10 |

Blood samples were drawn from the dogs for toxicokinetic analysis 0.5, 1, 2, 5, 10, and 24 hours after the last dose of gemifloxacin was administered. The dogs were observed for 2 more days without treatment.

**Results:** The goal of the study was to find the oral MTD that was not associated with vomiting for dogs from a new supplier. Toxicokinetic analysis would then be performed for each dog using the highest dose of gemifloxacin that did not cause vomiting.

Vomiting was observed in the male after the 250 and 300 mg/kg gemifloxacin doses on days 5 and 4, but not after the 200 mg/kg dose given on day 3. Thus, this dose was selected for the TK analysis. On day 8 (the TK profile day), however, one episode of vomiting occurred.

The female dog vomited after receiving ≥25 mg/kg, but not 10 mg/kg. After the 50 mg/kg dose was given, redness and thickening of the ears was observed in the female.

Neither body weight nor food consumption of either dog was affected by drug treatment.

Gemifloxacin was measurable in blood samples from both dogs throughout the 24 hour collection period. The investigators were not sure why this female was more sensitive to gemifloxacin-induced vomiting that has been previously observed with dogs- perhaps this individual was particularly sensitive.

**Toxicokinetics in a Male and Female Beagle Dog Following Oral Gemifloxacin Administration**

| | **Male (200 mg/kg)** | **Female (10 mg/kg)** |
|---|---|---|
| **Cmax (g/ml)** | 8.32 | 0.217 |
| **AUC (ghr/ml)** | 111 | 1.90 |
| **Tmax (h)** | 2.0 | 5.0 |

**Repeat-Dose Toxicity Studies:**

**SB 265805: 4-Day Intravenous Dose Toxicity Study in Rats** (SB Document No. SB-265805/RSD-100TSJ/4; Study No. D97666)

K. Parhar, R. Greenhill, A. Pritchard, S. Bullman (SmithKline Beecham, The Frythe, Welwyn, Herts, UK)

Report issued 11/11/98, amended 10/28/99

**Animals:** Male and female Sprague-Dawley rats (Crl:CD(SD)BR), 3/sex per dose group for the toxicity study, 3 females per gemifloxacin group for toxicokinetics, about 8 weeks of age, 375-395 g (males) and 222-262 g (females) on the first day of dosing, housed 3/cage by sex and dose group

**Diet:** SQC Expanded Ground Fine Rat and Mouse Maintenance Diet No. 1 and tap water were available *ad libitum*. Animals were fasted overnight after day 4 when urine samples were collected, prior to necropsy.

**Drug Dose and Route of Administration:** The doses of gemifloxacin used in this study were 10, 60, and 100 mg/kg (as free base). Gemifloxacin mesylate (Batch No. 02R1P1) was dissolved in sterile 0.9% saline and administered by slow IV bolus (1 ml/min) at a dose volume of 5 ml/kg. Vehicle control rats received saline only.

**Length and Conduct of Study:** The rats were treated daily for 4 days and sacrificed on the day after the last dose was given.

The animals were observed for clinical signs before dosing each day, immediately after the dose was administered, and at frequent intervals after dosing. Viability checks were made at the start and end of the working day. The rats were weighed daily and food and water consumption were recorded daily. Blood samples for clinical chemistry were taken on day 5. Urine was collected overnight on days 4-5. Tissues from control and high dose rats that were examined microscopically included adrenals, epididymides, heart, injection sites, kidneys, liver, lungs, ovaries, sternum, testes, thymus, and any gross lesions. The injection sites, kidneys, liver, sternum, and thymus from the low dose and mid dose rats underwent histopathologic evaluation as well. Kidney tissue was also examined by inferred and Raman microspectrometry.

Blood samples for toxicokinetics were drawn on day 4 of gemifloxacin administration 1, 10, and 30 minutes, and 2, 4, 8, and 24 hours after the end of injection. Plasma gemifloxacin levels were measured using [redacted] with [redacted] detection (lower limit of quantification was [redacted] g/ml).

**Results:** Unscheduled sacrifices occurred in the 60 and 100 mg/kg dose groups (2 rats in each, one high dose rat was a TK animal) due to marked necrosis of the tail at the injection sites.

Bruising of the tail was observed in 1/6 rats from the 10 mg/kg group (slight), 5/6 at 60 mg/kg (slight to moderate), and all rats in the 100 mg/kg group (slight to marked). Moderate to marked necrosis of the tail was seen in 4/6 rats at 60 mg/kg and 2/3 females at 100 mg/kg.

Most of the males in the 60 and 100 mg/kg groups lost weight. Dose-dependant decreases in food consumption were observed for both genders at 60 and 100 mg/kg (males 23-32% and females 11-19%). Increased water consumption (about 1.5-2 times

greater than control) was seen in both sexes of rat at 60 and 100 mg/kg.

A dose-related decrease in mean reticulocyte count was observed in both male and female rats at 60 and 100 mg/kg. Hematocrit, hemoglobin, and red blood cell counts were about 12-16% lower in 2 males and 1 female from the 100 mg/kg group than the lowest recorded values in the control group. White blood cell counts were also reduced in the males from the 60 and 100 mg/kg dose groups and females in the 100 mg/kg group (especially the lymphocytes in males).

In males, the plasma levels of urea and creatinine were up to 4.6 and 2.5 times the highest value from the control group in 1 rat from the 60 mg/kg group and all 3 from the 100 mg/kg group.

Urine volume in 1/2 surviving females from the 60 mg/kg group and both surviving females in the 100 mg/kg group was up to 4 times higher than the highest volume measured in the control group. As one would expect, _____ was lower than controls in these specimens.

Gross observations at necropsy included discoloration of the tail, with dose related severity, and slight enlargement of the kidneys in one male from the 100 mg/kg group. At the injection site, microscopic examination revealed epidermal and dermal necrosis in one male from the 10 mg/kg group and in all rats of both genders in the 60 and 100 mg/kg groups (severity was minimal to moderate). Tubular nephropathy (minimal to mild in females and mild to marked in males) was observed in all surviving rats from the 60 and 100 mg/kg dose groups and in 2 of the 4 premature decedents. Yellowish brown plugs were occasionally observed in the tubules. Pale yellow globular deposits were seen in unstained kidney sections of all 3 males in the 100 mg/kg dose group. Raman microspectrometry suggested that these deposits were gemifloxacin-related material. Minimal to mild pericholangitis was observed in 2/3 males and 1/2 females that were treated with 100 mg/kg gemifloxacin for the entire 4 days. Plugs were occasionally observed in the bile ducts. Minimal to mild hypocellularity was observed in the bone marrow in the sternum in 1 male from the 60 mg/kg group and 2 rats from each gender in the 100 mg/kg group. Minimal hypocellularity of this tissue was also seen in 2/4 premature decedents. Minimal to moderate atrophy of the thymus was observed in most animals from the 2 highest dose groups and the investigators believed that this might have been due to stress from the lesions at the injection site or the kidneys.

**Mean Toxicokinetic Parameters in Female Rats Following Multiple IV Doses of Gemifloxacin**

| Dose (mg/kg) | Cmax (g/ml) | $AUC_{0\text{-}24\,h}$ (ghr/ml) |
|---|---|---|
| 10 | 23.0 | 14.2 |
| 60 | 108 | 62.7 |
| 100 | 96.8* | 98.6* |

*N=2

Gemifloxacin could be detected in the plasma for the whole 24 hour sampling

period. Intra-animal variability was relatively high for Cmax, but it was lower for AUC.

Gemifloxacin was associated with a dose-related decrease in food consumption and body weight loss when given at 60 or 100 mg/kg/day for 4 days. Drug-related histopathologic changes included hypocellularity of bone marrow in the sternum (associated with decreased reticulocytes and white blood cells in the peripheral blood), renal tubular nephropathy (probably related to drug-associated material found in the kidney and associated with increased plasma urea and creatinine levels), and pericholangitis (possibly related to plugs in the bile ducts that may contain drug-associated material). Bruising and necrosis of the injection site was observed as early as day 2 of dosing in the 100 mg/kg rats and led to some animals being sacrificed early for humane reasons. Excluding one male with an injection site lesion, the NOAEL for gemifloxacin administered IV daily for 4 days was 10 mg/kg.

**SB 265805: 14-Day Intravenous Dose Toxicity Study in Male Rats** (SB Document No. SB-265805/RSD-100V33/2; Protocol No. G98545)

J.M. Birmingham, R. Greenhill, A. Pritchard, J.N. Bullman (SmithKline Beecham, The Frythe, Welwyn, Herts, UK)

Report issued 11/24/98, amended 8/6/99; UK, US, and OECD GLP

Vol. 1.5.010

**Animals:** Male Sprague-Dawley rats (Crl: (IGS) CD BR), 10 per dose group for the toxicity study, 3 per gemifloxacin group for toxicokinetics, about 10 weeks of age, 328-417 g on the first day of dosing, housed 5 or 3 per cage by dose group

**Diet:** SQC Rat and Mouse Maintenance Diet No. 1 and filtered tap water were available *ad libitum*. Animals were fasted overnight prior to necropsy while urine samples were collected.

**Drug Dose and Route of Administration:** The doses of gemifloxacin used in this study were 2, 10, 20, and 40 mg/kg (as free base). Gemifloxacin mesylate (Batch No. EF03-02R1P1) was dissolved in sterile 0.9% saline and administered at a dose volume of 10 ml/kg over 30 minutes. Vehicle control rats received saline only.

**Length and Conduct of Study:** The rats were treated daily for up to 15 days and sacrificed on the day after the last dose was given.

The animals were observed for clinical signs before dosing each day, immediately after the dose was administered, and 1-4 hours after dosing. Viability checks were made at the start and end of the working day. The rats were weighed daily and food and water consumption were recorded daily. Ophthalmoscopy was conducted prior to dosing on all rats and on day 11 of treatment for control rats and surviving animals in the 20 and 40

mg/kg groups. Blood samples for clinical chemistry were taken on day 14 or 15. Urine was collected overnight prior to the day of necropsy. The tissues collected at necropsy from all rats and examined microscopically in the control, 20, and 40 mg/kg rats are listed in the table at the end of the review. The injection sites and kidneys from rats in all treatment groups were examined. The Harderian gland, larynx, nasal turbinates, rectum, skull, and tongue were harvested and preserved, but not examined. The left femur with stifle joint was taken only from the control, 20, and 40 mg/kg rats.

Blood samples for toxicokinetics were drawn on day 1 and 14 of gemifloxacin administration immediately after infusion, and 1, 1.5, 2, 4, 8, and 24 hours after the end of infusion. Plasma gemifloxacin levels were measured using ——with ⊢ detection (lower limit of quantification was —— g/ml).

**Results:** In the 40 mg/kg group, 5 rats assigned to the toxicity part of the study and one rat assigned to the TK part of the study were sacrificed early (days 4-9) due to the poor condition of their tails at the injection sites. One rat from the 20 mg/kg group was also sacrificed early for the same reason. One TK rat from the 10 mg/kg group died during a blood draw on day 14.

Bruising at the injection site was observed in 1 rat from the 10 mg/kg group, 6/10 rats at 20 mg/kg, and 9/10 rats at 40 mg/kg group. Some of the rats from the 40 mg/kg group began to exhibit bruising as early as day 1. As the condition of the tails deteriorated, swelling and a blackened area indicative of necrosis were observed in several rats from the 20 and 40 mg/kg groups and reddening, scabbing, ulceration, and erosion of the tail surface were also observed grossly in some high dose rats.

Group mean body weight gain in the 40 mg/kg rats was significantly less (by 88%) than control during the first week of the study and mean food consumption was reduced by about 20%. The surviving 40 mg/kg animals gained weight at a similar rate as the control group during the second week and their food consumption was also similar to controls. Mean water consumption in the 40 mg/kg rats was increased by 78-107% compared to control throughout the dosing period. In the 20 mg/kg group, mean water consumption was about 12% greater than control during the study.

No drug-related ophthalmic changes were observed. Group mean hematocrit, hemoglobin, and red cell count was about 10% lower (a slight, but statistically significant reduction) than controls in the 40 mg/kg group. No reduction in white blood cells or reticulocytes (as was seen when rats received IV gemifloxacin doses $\geq$60 mg/kg for 4 days) was observed in this study. The investigators thought that the slight reduction in red cell parameters coupled with a small reduction in the mean plasma albumin concentration (see below) observed in the 40 mg/kg dose group might indicate an increase in plasma volume, perhaps secondary to the increased water consumption observed in these rats.

The mean serum urea level was about 40% higher in the 40 mg/kg group than control and mean creatinine was about 11% higher than control in the 40 mg/kg rats. Serum cholesterol was slightly higher (up to 35%) in the 20 and 40 mg/kg rats than control. Mean plasma albumin concentration was about 6% lower in the 40 mg/kg group than control. Some other statistically significant differences in serum chemistry between

treated and control rats were so small that their biological significance is questionable.

Mean urine volume collected from the 40 mg/kg rats was about 43% higher than control (though this increase was not statistically significant due to variability) and the osmolality was about 38% lower than control, perhaps secondary to the increased water consumption in this gemifloxacin treatment group. In contrast, the urine output was about 34% less than control in the 20 mg/kg group and the urinary osmolality was about 41% greater than control, despite the slightly increased water consumption observed in the 20 mg/kg rats. In both the 40 and 20 mg/kg groups, creatinine clearance was decreased in a dose-related manner (up to 22%) and urinary pH in these groups was about 0.5 pH units lower than control. Urinary sodium excretion was reduced compared to controls in the 20 and 40 mg/kg groups by about 30-40% and potassium excretion was increased by about 30% in the 40 mg/kg group. The urinary excretion of chloride was reduced by about 40% for the 20 mg/kg group. Red-brown spherical crystals with rough surfaces were observed in urine specimens from 4/9 rats in the 20 mg/kg group and 5/5 rats in the 40 mg/kg group that survived until scheduled sacrifice. Other "atypical" crystals (described in the report as "including rosettes, prisms, and rectangular plates") were seen in the urine of 8/10 rats in the 10 mg/kg group and in 3/9 rats in the 20 mg/kg group.

Absolute and relative mean kidney weights in the 40 mg/kg rats were greater than controls. Depressions on the kidney surface were observed during gross necropsy in 4 animals from this dose group. Histopathologic analysis of the kidney showed tubular nephropathy in 1/10 rats in the 10 mg/kg group, 7/10 at 20 mg/kg, and all 40 mg/kg rats (premature decedents included). Changes in the kidney included basophilia of the tubular epithelial epithelium of the distal nephron, tubular dilatation, and interstitial inflammatory cell infiltration. The severity of the kidney findings was dose-related, ranged from mild to moderate, and extended to the proximal nephron in the most severely affected rats. Yellow/brown plugs were seen in the distal tubules in several rats in the 20 mg/kg group and in all rats at 40 mg/kg.

Discoloration at the injection site in several rats from the 20 and 40 mg/kg groups was another gross observation made during necropsy. Microscopic examination of this site revealed an increase in the amount of inflammatory cell infiltration in rats from these dose groups, compared to control. Necrosis (epidermal and dermal) was also seen at the injection site in some of the 20 and 40 mg/kg rats.

Toxicokinetics were performed using only the data collected on day 14. The concentrations of gemifloxacin in plasma samples collected on day 1 did not appear to be accurate (highest concentrations did not generally occur at the end of infusion and the concentrations rose and fell during the sampling period- not consistent with what should occur following an IV infusion). No results are presented for the 40 mg/kg dose group since they could not be bled on day 14 of dosing due to poor tail condition. Gemifloxacin could be quantified in the plasma of rats given 2 mg/kg up to 8 hours after administration and in plasma from the 10 and 20 mg/kg groups for the whole 24 hour sampling period.

**Toxicokinetic Parameters in Rats After 14 Consecutive Daily IV Doses of Gemifloxacin**

| Dose (mg/kg/day) | Cmax (g/ml) | $AUC_{0-t}$ (ghr/ml) |
|---|---|---|
| 2 | 1.33 ± 0.57 | 1.41 ± 0.42 |
| 10 | 3.97 ± 0.55 | 6.49 ± 0.39 |
| 20 | 17.2 ± 12.8 | 22.1 ± 7.71 |

N=3 in the 2 mg/kg group, N=2 in the 10 and 20 mg/kg groups

Approximately half of the rats given 40 mg/kg/day of gemifloxacin and one rat given 20 mg/kg/day via the tail vein had to be sacrificed before the end of the 14 day study period due to the poor condition of their tails. The injection sites of many rats in these dose groups (including premature decedents and survivors) were discolored, with inflammatory cell infiltration and epidermal/dermal necrosis observed microscopically. Decreased food consumption and body weight gain were seen during the first week of dosing with 40 mg/kg of gemifloxacin. Increased water consumption was seen at 40 mg/kg and signs of a slightly increased plasma volume were observed in the rats from this dose group (slight decreases in red cell parameters and plasma albumin concentration). A smaller increase in water consumption was also observed at 20 mg/kg. In contrast to the previous 4 day IV study in rats (which used doses as high as 60 and 100 mg/kg/day) hypocellularity of bone marrow in the sternum (associated with decreased reticulocytes and white blood cells in the peripheral blood) and pericholangitis were not observed in the current 14 day study. Renal tubular nephropathy was observed in 1/10 rats in the 10 mg/kg group, 7/10 at 20 mg/kg, and all 40 mg/kg rats. Microscopic changes in the kidneys included basophilia of the tubular epithelial epithelium of the distal nephron, tubular dilatation, and interstitial inflammatory cell infiltration. Plugs in the distal tubules of rats from the 20 and 40 mg/kg dose groups appeared similar to those seen in the previous 4 day study that were shown to contain drug-related material. Changes in serum chemistry in the 40 mg/kg group (increased urea and creatinine levels) and decreased urinary creatinine clearance were also indicative of renal toxicity. Drug-related crystal nephropathy has been seen in rats when other quinolones were administered. The NOAEL for gemifloxacin administered IV daily for 14 days was 2 mg/kg.

**SB 265805: 1-Month Intravenous (1-Hour Infusion) Dose Toxicity Study in Rats Followed by a 28-Day Off-Dose Period** (SB Document No. SB-265805/RSD-100XTB/2; Protocol No. G98642; CRO Study Code 802/518)

---

Report dated 6/23/99, amended 11/29/99, OECD and US GLP

Vol. 1.5.011

**Animals:** Male and female Sprague-Dawley rats (Crl:CD(SD)BR), 15/sex per dose group for the toxicity study (with 5 per dose group used to assess reversibility of any drug-related effects), 6 per gemifloxacin group for toxicokinetics; about 10 weeks of age, 327-428 g (males) and 219-275 g (females) on the first day of dosing

**Diet:** Pelleted complete Rat Diet A04-C10 ( [redacted] , and filtered tap water were available *ad libitum*. Food and water were removed overnight prior to necropsy while urine samples were collected.

**Drug Dose and Route of Administration:** The doses of gemifloxacin used in this study were 2, 10, and 20 mg/kg (as free base). Gemifloxacin mesylate (Batch No. FNS-A-08C) was dissolved in sterile 0.9% saline and administered at a dose volume of 10 ml/kg over 60 minutes. Vehicle control rats received saline only. The infusions were performed using an indwelling catheter implanted into the posterior vena cava of each animal via the femoral vein. Between drug treatments, saline was continuously infused through the catheters at 0.4 ml/h.

**Length and Conduct of Study:** The rats assigned to the toxicity portion of the study were treated daily for 28 days and sacrificed on the day after the last dose was given or maintained for another 28 days with no drug treatment if they were part of the subgroup used to determine reversibility of drug-related effects.

The animals were observed for clinical signs before dosing each day, about midway through the daily drug infusion, at the end of infusion, and 1-6 hours after dosing. The observation made midway through infusion was eliminated after the first week of dosing was completed. During the recovery period, rats were observed once daily for clinical signs. Viability checks were made at the start and end of the working day throughout the entire study. The rats were weighed, and food and water consumption were recorded twice weekly. Ophthalmoscopy was conducted prior to dosing on all rats assigned to the toxicity portion of the study and at the end of treatment for control rats and surviving animals in the 20 mg/kg group. Blood samples for clinical chemistry and hematology were taken on the day before scheduled necropsy. Additional samples for hematology were taken from the rats assigned to the recovery portion of the study 2 weeks and 4 weeks after the end of treatment. Urine was collected overnight prior to the day of necropsy after the rats received 20 ml/kg of tap water by oral gavage. Food and water were removed from cages during the urine collection period. The tissues collected at necropsy from all rats and examined microscopically in the control and 20 mg/kg rats that were sacrificed at the end of the dosing period and all premature decedents are listed in the table at the end of the review. Transverse sections were prepared from one testes of each male from the control and 20 mg/kg gemifloxacin groups that died during treatment or were sacrificed after 4 weeks of treatment. These were stained with Periodic Acid Schiff reagent and hematoxylin and sent to SKB for stage-dependent evaluation of spermatogenesis (slides from 5 males in each of these groups were examined). In

addition, bone marrow was examined microscopically *in situ* in the sternum and the stifle joint of the right femur was examined microscopically. The injection sites, kidneys, and any gross lesions from rats in all treatment groups were examined microscopically (unless histopathologic examination of the gross lesions was considered unnecessary by the study pathologist). All kidneys and injection sites were also examined under polarized light for the presence of crystals. The Harderian gland, cervix, larynx, nasal turbinates, preputial gland, clitoral gland, rectum, skull, tongue, and bone marrow smears were harvested and preserved, but not examined.

Blood samples for toxicokinetics were drawn on days 1 and 27 of gemifloxacin administration immediately after infusion, and 1.5, 2, 6, 10, and 24 hours after the end of infusion (3 rats per sex for each time point). Plasma gemifloxacin levels were measured using —— with ——detection (lower limit of quantification was —— g/ml).

**Results:** No gemifloxacin-related mortality or clinical signs were observed during the study.

For the first 5 days of dosing, the rats in the 20 mg/kg group had a 30-40% reduction in mean body weight gain compared to controls and they also had a slight decrease in food consumption (about 8-9%). For the remainder of the study, however, body weight gain and food consumption in the 20 mg/kg group was similar to control. At the end of the dosing period, there was no significant difference between the body weight of the control rats and any drug treated group. During the recovery period, body weight gain in the 20 mg/kg group was about 30-40% greater than controls; food consumption was generally similar to control or slightly higher. Water consumption was 1.2-1.8 times higher in the male 20 mg/kg rats than controls during the dosing period. It was similar to control (or just slightly higher) during the recovery period. Water consumption in all groups of gemifloxacin-treated female rats was similar to control.

No drug-related ophthalmic changes were observed.

The males in the 20 mg/kg group had slightly (4-5%), but statistically significantly lower mean hemoglobin, hematocrit, and red blood cell counts than controls. These parameters were about 3% lower than controls in female rats from the 20 mg/kg group, but that difference was not statistically significant. Neither difference is likely to be of biological significance, but the reductions are consistent with observations made in previous IV rat studies. After 2 weeks of recovery, the 20 mg/kg males still had a statistically significant reduction (4%) in mean hemoglobin concentration than controls and the 20 mg/kg females had statistically significant reductions in (5%) mean hemoglobin concentration and hematocrit compared to controls. At the end of the 4 week recovery period, no statistically significant differences in any of these parameters were observed. No other drug-related changes in hematologic parameters were seen.

Plasma protein and globulin concentrations in the 20 mg/kg males were 3% and 11% less than controls, statistically significant reductions. Coupled with the reduction in the red cell parameters above and the increased water consumption, this may indicate a slightly increased plasma volume in the 20 mg/kg males. No other changes in serum chemistry appeared to be related to gemifloxacin treatment.

Urinary pH tended to be lower in specimens from gemifloxacin-treated rats (pH

6-7) than controls (pH 7-8), and the incidence of this reduction was dose-related. There were fewer bacteria in urine from rats in the gemifloxacin groups. No abnormal crystals were observed in the urine from drug-treated rats. Mean urine volume was 19% lower than control in 20 mg/kg females (a statistically significant reduction), but 13% higher than control in 20 mg/kg males (not statistically significant, but it is consistent with their increased water consumption). The 20 mg/kg males had increased potassium excretion compared to controls (about 30% higher, a statistically significant increase), perhaps secondary to the tubular nephropathy observed in this group.

Although there were some slight changes in group mean organ weights in high dose rats compared to controls (e.g., increased relative spleen weights in males after 4 weeks of dosing; decreased epididymal weights in males after the recovery period; decreased relative thymus weights in females after 4 weeks of dosing, but increased relative thymus weights in females after the recovery period), none appeared related to a drug and there were no microscopic changes in any of these tissues. Stage-dependent analysis of spermatogenesis revealed no effect of gemifloxacin.

The injection sites of some rats (mostly males in the 10 and 20 mg/kg groups, but also 2 females in the 10 mg/kg group) demonstrated induration, raised areas, nodules, or masses- these were shown to be thrombi. These were generally associated with a reaction in the wall of the vein and often contained yellow/brown plugs. Microscopic examination of the injection sites revealed thrombi in some rats from all treatment groups, but the incidence was higher in the 20 mg/kg gemifloxacin group. The yellow/brown plugs seen in the thrombi from rats in the 10 and 20 mg/kg groups were trapped stellate crystals (probably drug-related material). The quantity of crystals deposited at the injection site correlated with the severity of injection site reactions in the wall of the vein. Histopathological changes at the injection site included vasculitis, perivasculitis, and granulomas. Thrombi with crystals and injection site reactions were still seen after the 4 week recovery period in the 20 mg/kg group.

Microscopic examination of the kidneys of rats sacrificed at the end of the treatment period showed slight to moderate tubular nephropathy with tubular dilatation in all males and 1/10 females from the 20 mg/kg group and 4/10 males in the 10 mg/kg group. Crystals were seen in the lumen of the distal tubules in 9/10 males in the 20 mg/kg group and 4/10 males in the 10 mg/kg group. Crystals were also observed in the renal pelvis of 3/10 males and 4/10 females in the 20 mg/kg group and 2/10 males at 10 mg/kg. Basophilic tubules in the kidneys of male rats were observed at a higher incidence in the 10 and 20 mg/kg gemifloxacin groups than in controls and interstitial inflammatory cell infiltration was present at a higher incidence at 20 mg/kg. Other apparent drug-related changes in the kidney were papillary epithelial hyperplasia seen in the 1/10 males at 10 mg/kg and 6/10 males at 20 mg/kg, and an increased incidence of mineralization in females treated with 20 mg/kg of gemifloxacin. Acute pyelonephritis was seen in one of the 20 mg/kg males. After the 4 week recovery period, depressed areas of the kidneys were seen in 3/5 male rats at 20 mg/kg. Microscopic examination of these areas revealed changes (a focal area of basophilic tubules associated with interstitial inflammatory cell infiltration and an area of chronic interstitial nephritis) that may have

been associated with scarring which could have been caused by crystal deposition.

Gemifloxacin could be quantified in plasma up to 6 hours after the 2 mg/kg dose and up to 10 hours after the 10 and 20 mg/kg doses. There appeared to be no sex-related differences in toxicokinetic parameters. Accumulation of the drug did not occur at the dose levels used in this experiment. The plasma concentration of the (+) enantiomer was usually slightly higher than that of the (-) enantiomer; this has been observed in other studies. The plasma and AUC values for gemifloxacin increased in a dose-proportional manner.

**Mean Cmax and AUC Values for the (+) and (-) Enantiomers of Gemifloxacin In Rats After Intravenous Administration**

| | | | 2 mg/kg | | 10 mg/kg | | 20 mg/kg | |
|---|---|---|---|---|---|---|---|---|
| | | | M | F | M | F | M | F |
| Day 1 | Cmax (g/ml) | + | 0.206 | 0.203 | 1.08 | 1.14 | 2.54 | 2.23 |
| | | - | 0.179 | 0.164 | 0.892 | 0.911 | 2.18 | 1.90 |
| | | T | 0.385 | 0.367 | 1.97 | 2.05 | 4.72 | 4.13 |
| | $AUC_{0-t}$ (gh/ml) | + | 0.332 | 0.215 | 1.68 | 2.16 | 4.43 | 4.31 |
| | | - | 0.298 | 0.176 | 1.55 | 1.84 | 4.18 | 3.94 |
| | | T | 0.630 | 0.391 | 3.23 | 4.00 | 8.61 | 8.25 |
| Day 27 | Cmax (g/ml) | + | 0.221 | 0.237 | 0.937 | 1.21 | 2.27 | 2.30 |
| | | - | 0.186 | 0.184 | 0.774 | 0.942 | 1.92 | 1.90 |
| | | T | 0.407 | 0.421 | 1.71 | 2.15 | 4.19 | 4.20 |
| | $AUC_{0-t}$ (gh/ml) | + | 0.360 | 0.373 | 1.79 | 2.34 | 5.07 | 4.72 |
| | | - | 0.286 | 0.311 | 1.63 | 2.00 | 4.70 | 4.10 |
| | | T | 0.646 | 0.684 | 3.42 | 4.34 | 9.77 | 8.82 |

T=total

Intravenous infusion with 10 or 20 mg/kg/day of gemifloxacin for 28 days via an indwelling catheter was associated with an increased incidence of thrombi and increased severity of injection site reactions compared to controls. The severity of the injection site reactions was associated with crystal deposition. Decreased food consumption and body weight gain were seen during the first several days of dosing with 20 mg/kg of gemifloxacin, but both were similar to controls for the rest of the treatment period.